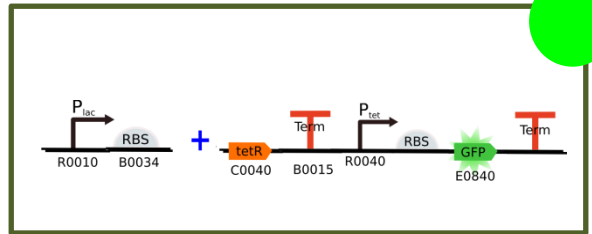


## Assembly:

Pl\_Term



## 1<sup>st</sup> Day:

### EXSP Digestion (see **Enzymatic Digestion Protocol**)

	Part	Size	ng/μl
1	Pl_RBS	212 bp	102.5
2	tet_E0840	1745 bp	205.0

	Volume to 1,0 μg (μl)	Buffer 10x (μl)	BSA (μl)	Enzyme 1	Volume (μl)	Enzyme 2	Volume (μl)	H <sub>2</sub> O to 20μl (μl)
1	6.2	2 (M)	-	S	1	P	1	9.8
2	9.2	2 (M)	2	X	1	P	1	4.8

Final Plasmid	Resistance
pSB1A2	Ampicillin

## Gel purification

- See PureLink® Quick Gel Extraction Kit Invitrogen™ manual
- Quantify digestion products

Parts	ng/μl
Pa_RBS	8.3
tet_E0840	6.7

**Obs:** 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

## Ligation (see **Ligation Protocol**)

Part containing the plasmid	<b>Pa_RBS</b>	67
Insert	tet_E0840	18
10x T4 DNA Buffer	3	
T4 DNA ligase 1u	0.4	
H2O to 30μl	2.6	

**Obs:** To determinate the amount of DNA necessary we used the following equation

$$\text{Insert ng} = \text{plasmid ng} \times \text{insert bp} / \text{plasmid bp} \times \text{insert: plasmid ratio}$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium.
- Prepare glycerol 40%

### **2<sup>nd</sup> Day:**

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Transformation (see **Transformation Protocol in Escherichia coli DH5-α**)

- Organism: E. coli DH5-α
- Selection: Ampicillin

### **4<sup>th</sup> Day:**

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Confirmation with NotI.